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Major Article

High rate of detection of OXA-23-producing *Acinetobacter* from two general hospitals in Brazil

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Abstract

Introduction: In recent decades, the prevalence of carbapenem-resistant *Acinetobacter* isolates has increased, and the production of oxacillinase (OXA)-type carbapenemases is the main mechanism underlying resistance. We evaluated OXA production from 114 *Acinetobacter* isolates collected between March and December 2013 from different clinical specimens of patients in two hospitals (Hospital 1 [n = 61] and Hospital 2 [n = 53]) located in Niterói, Rio de Janeiro, Brazil. We also evaluated the genetic diversity of OXA-producing isolates. **Methods**: All the isolates were identified through the automated system Vitek II and matrix-assisted laser desorption ionization-time of flight mass spectrometry MALDI-TOF MS as belonging to the *A. baumannii-A. calcoaceticus* complex. Antimicrobial susceptibility profiles were verified through agar diffusion tests. The presence of OXA-encoding genes was confirmed by PCR. The genetic diversity of isolates positive for carbapenemase production was analyzed through pulsed-field gel electrophoresis. **Results**: There was a high rate of resistance to carbapenems in the isolates (imipenem: 96%; meropenem: 92%) from both hospitals. Moreover, a high percentage (95.6%) of OXA-23-positive isolates was observed for both hospitals, indicating that this was the main mechanism of carbapenem-resistance among the studied population. In addition, most isolates (96.5%) were positive for $bla_{\text{OXA-51}}$. A high genetic diversity and a few major genotypes were found among the OXA-23-positive isolates analyzed. Only intra-hospital dissemination was observed. **Conclusions**: The elevated dissemination of $bla_{\text{OXA-23-like}}$ observed among *Acinetobacter* isolates from both the studied hospitals highlights the need for continuous epidemiological surveillance in these institutions.

Keywords: *Acinetobacter* spp. Carbapenem-resistance. OXA-23.

INTRODUCTION

A. baumannii-A. calcoaceticus complex includes opportunistic pathogens affecting severely ill patients. The choice of treatment for serious infections caused by *Acinetobacter* is frequently based on the use of carbapenems[1]. However, carbapenem resistance among clinical isolates of *Acinetobacter* spp. has been reported worldwide. In addition, these isolates may have concomitant resistance to most conventional antimicrobial agents and cause difficulty in treating infections, and leave few treatment options[2].

Different mechanisms can confer resistance to carbapenems in *Acinetobacter*, such as decreased permeability of the outer membrane, increased expression of efflux pumps, changes in the affinity of penicillin-binding proteins, and the production of carbapenemases. Among them, the production of carbapenemases, such as more frequent Class D beta-lactamases, also called oxacillinases (OXAs), less frequent Class B, also known as metallo-beta-lactamases (including IMP, VIM, SIM, and NDM-1 types) and Class A (of KPC or GES type) is the most important carbapenem resistance mechanism[3-5].

The main groups of OXA-type carbapenemases identified in *A. baumannii* are OXA-23-like, OXA-24/40-like, OXA-58-like, OXA-143-like, and OXA-235-like groups and are composed

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of acquired enzymes and OXA-51-like group, which encodes a chromosomal intrinsic OXA in *A. baumannii*. This intrinsic OXA may be super-expressed and associated with carbapenem resistance[6,7]. In Brazil, carbapenem-resistant *Acinetobacter* usually produce OXA-23, followed by OXA-143[8,9].

Thus, we aimed to characterize carbapenem-resistant *Acinetobacter* isolates obtained from two health institutions located in the city of Niterói, Rio de Janeiro State, Brazil, evaluated the production of OXAs, and determined their genetic relationship.

METHODS

Bacterial Isolates

One hundred and fourteen *Acinetobacter* isolates were obtained from patients at two general hospitals located in Niterói; Hospital 1 (a 290-bed public university teaching hospital; n = 61) and Hospital 2 (a 201-bed tertiary private hospital; n = 53) from March to December 2013.

The most frequent sites for the collection of these specimens were the lower respiratory tract containing secretions (48; 42.1%) such as tracheal aspirate (40; 83.3%), bronchoalveolar lavage (6; 12.5%), sputum and pleural fluid (1 each; 2.1% each), the blood (23; 20.2%), the urine (12; 10.5%), and catheter tip (9; 7.9%). Other sources represented 11.4% (n = 13) and the origin of nine (7.9%) isolates could not be determined.

Identification and antimicrobial susceptibility testing

The isolates were previously identified using VITEK-2 automated system (BioMerieux, Craponne, France) as *A. baumannii-A. calcoaceticus* complex at the microbiology laboratories of each of the two health institutions. The final identification of *A. baumannii-A. calcoaceticus* complex was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry on a Maldi Biotyper platform (Bruker Daltonics, Germany).

The disc diffusion method was used to evaluate susceptibility to the following antimicrobials agents according to Clinical and Laboratory Standards Institute guidelines[10]: amikacin (30 μg), ampicillin/sulbactam (10/10 μg), ceftazidime (30 μg), cefepime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), sulfamethoxazole/trimethoprim (23.75 μg/1.25 μg), tetracycline (30 μg), and tobramycin (10 μg). Minimum inhibitory concentration (MIC) values for imipenem were determined by Etest (AB Biodisk, Solna, Sweden).

Molecular investigations

The isolation of bacterial DNA was accomplished through thermal lysis, according to the methodology proposed by Schuenck with modifications[11]. PCR was used to detect the genes encoding OXA-like carbapenemases: $bla_{\text{OXA-23-like}}$[12], $bla_{\text{OXA-24-like}}$[12], $bla_{\text{OXA-58-like}}$[12], $bla_{\text{OXA-51-like}}$[12], and $bla_{\text{OXA-143}}$[13].

The analysis of genetic diversity was investigated through pulsed-field gel electrophoresis (PFGE), as described in a previous study[14] using *Apa*I restriction enzyme (Invitrogen, São Paulo, Brazil). For PGFE analysis, 38 OXA-23 producing isolates from patients in both hospitals (n = 25, Hospital 1 and n = 13, Hospital 2) were included. Only one isolate per patient was considered. Seven representative strains of the two major clonal groups detected in Hospital 1 (genotype A, n = 4 and genotype B, n = 3) in a previous study, with OXA-23-producing *Acinetobacter* isolates from the year 2007 to 2009 (LL Corrêa, CRV Mendonça-Souza; data unpublished) were also included in the analysis for comparison purposes. The patterns obtained were analyzed using BioNumerics v7.6 software (Applied Maths, Belgium). The comparison of the banding patterns was accomplished through the unweighted pair-group method with arithmetic mean, with a tolerance and optimization of 1.0% using the Dice correlation coefficient. A similarity cut-off of 80% was used for clustering isolates in the same genotype.

Ethics

This study was approved by the Human Research Ethics Committee of the School of Medicine of Universidade Federal Fluminense, under protocol number 276.895.

RESULTS

The results of antimicrobial susceptibility testing showed high resistance rates of most antimicrobials tested. Among Hospital 1 isolates, the highest resistance rates were observed with imipenem and meropenem (92%), followed by ciprofloxacin (90%), whereas among Hospital 2 isolates, the highest resistance rates were seen with imipenem and ciprofloxacin (100%), followed by meropenem (92%). The highest rates of susceptibility were observed with amikacin (3/114; 2.6%) and tetracycline (4/114; 3.5%) in both hospitals. In general, the resistance rates verified in Hospital 2 were higher than those in Hospital 1, except for trimethoprim/sulfamethoxazole and tobramycin. In addition, given the number of carbapenem-resistant isolates from both hospitals, 82.1% isolates were resistant to at least one agent of two more other classes of antimicrobials tested, indicating multidrug resistance.

According to the PCR results, 96.5% (110/114) of isolates presented $bla_{\text{OXA-51-like}}$, originally intrinsic to *A. baumannii*, and 95.6% (109/114) carried $bla_{\text{OXA-23-like}}$. No isolate was positive for the other OXA-encoding genes investigated.

All 109 $bla_{\text{OXA-23-like}}$/$bla_{\text{OXA-51-like}}$-positive isolates were resistant to at least one of the carbapenems tested, except for one isolate (from Hospital 1) that had an imipenem MIC of 0.38 μg/mL. One isolate positive to only $bla_{\text{OXA-51-like}}$ (from Hospital 2) was resistant to 9 of 11 antibiotics tested including the two carbapenems tested, and was susceptible to only tetracycline and ceftazidime.

PFGE analysis of the 25 isolates from Hospital 1 revealed a polyclonal pattern, but with three major genotypes: A (n = 5; 20%), C (n = 4; 16%), and D (n = 4; 16%). The five isolates belonging to genotype A clustered with four genotype A representative strains, detected in a previous study, with a coefficient of similarity of ≥ 80%. None of the analyzed isolates was related to the three representative strains belonging to genotype B. (**Table 1**).

For Hospital 2, one predominant genotype, named N, included six isolates (46.2%), clustered with 80% similarity

TABLE 1: Characteristics of 38 OXA-23-producing *Acinetobacter* isolated in Niterói city, Brazil.

Pulsotype	Isolate	Hospital	Date of Isolation	Clinical specimen	Antimicrobial resistance profile[a]
A	CS30122	1	04/30/13	Blood	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30134	1	05/14/13	Urine	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30151	1	05/24/13	Liquor	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30153	1	05/26/13	Liquor	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30246	1	09/02/13	Blood	IMP; MER; CPM; SUT
C	CS30105	1	03/25/13	Catheter tip	IMP; MER; GEN; CIP; SUT
	CS30115	1	04/19/13	Catheter tip	IMP; MER; CIP; SUT
	CS30121	1	05/01/13	Blood	IMP; MER; GEN;TOB; CIP; SUT
	CS30176	1	06/20/13	Urine	IMP; MER; CPM; CIP; SUT
D	CS30279	1	10/01/13	BAL[b]	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30285	1	10/07/13	Blood	IMP; MER; CPM; GEN; TOB; CIP; SUT
	CS30289	1	11/01/13	Blood	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30292	1	11/02/13	Blood	IMP; MER; CIP; SUT
E	CS30321	1	11/08/13	Urine	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30322	1	11/23/13	Blood	IMP; MER; CAZ; CIP; SUT
F	CS30118	1	04/19/13	Tracheal secretion	IMP; MER; CAZ; CIP
G	CS30278	1	09/30/13	Blood	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30282	1	09/19/13	Skin biopsy	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
H	CS30280	1	09/30/13	Urine	IMP; MER; CIP
	CS30283	1	09/30/13	Blood	IMP; MER; CIP
I	CS30204	1	07/09/13	Blood	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
J	CS30177	1	06/21/13	Catheter tip	IMP; MER; CPM; SUT; CIP
K	CS30323	1	12/13/13	Urine	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
L	CS30284	1	10/14/13	Blood	IMP; MER; CPM; CIP; SUT
M	CS30174	1	06/07/13	Renal perfusion fluid	none
N	CS30104	2	03/25/13	Catheter tip	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30162	2	05/25/13	Blood	IMP; MER; CIP; SUT
	CS30199	2	07/04/13	Abdominal fragment	IMP; MER; CAZ; CPM; GEN; CIP; SUT
	CS30195	2	07/29/13	BAL	IMP; MER; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30213	2	07/30/13	Blood	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
	CS30214	2	07/30/13	BAL	IMP; MER; ASB; CAZ; CPM; GEN; TOB; CIP; SUT
O	CS30111	2	05/20/13	BAL	IMP; MER; ASB; CPM; CIP; SUT
P	CS30182	2	06/01/13	Catheter tip	IMP; CAZ; CPM; GEN; CIP
Q	CS30197	2	07/15/13	BAL	IMP; MER; CAZ; GEN; TOB; CIP; SUT
R	CS30254	2	09/09/13	Tracheal secretion	IMP; MER; ASB; CAZ;CPM; GEN; CIP; SUT
S	CS30258	2	09/11/13	Rectal swab	IMP; MER; CIP; SUT
T	CS30136	2	04/29/13	Pleural fluid	IMP; MER; CAZ; CPM; CIP
U	CS30198	2	07/02/13	Catheter tip	IMP; MER; CIP

[a] **IMP**: Imipenem, **MER:** Meropenem, **ASB:** Ampicillin/Sulbactam, **CAZ:** Ceftazidime, **CPM:** Cefepime, **GEN:** Gentamicin, **TOB:** Tobramycin, **CIP:** Ciprofloxacin, **SUT:** Sulfamethoxazole/Trimethoprim; [b]:**BAL:** Bronchoalveolar lavage.

(**Table 1**). Inter-hospital dissemination was not observed in our study.

DISCUSSION

This study described the resistance profiles and genetic relatedness of carbapenem-resistant *A. baumannii* complex isolates collected from patients in two health institutions located in Niterói, Rio de Janeiro, Brazil.

Carbapenem resistance is a serious problem in the treatment of infections caused by *Acinetobacter*, since these antibiotics are considered as one of the best therapeutic options for the treatment of severe infections caused by *Acinetobacter*. A study from SENTRY showed an increase in the proportion of carbapenem-resistant *Acinetobacter* spp., with the rate of 70% in 2008-2009[15]. Another study from Niterói also verified carbapenem-resistance rate of 70% among *Acinetobacter* isolated in 2007-2009[16]. In the present study, an even higher resistance rate to carbapenems was observed (> 90%) comparable to the results of other recent Brazilian studies that also showed carbapenem resistance rate > 90% among *A. baumannii* isolates[17,18], highlighting an increasing trend in the dissemination of carbapenem-resistant *Acinetobacter* isolates in the last few years.

We noted that carbapenem resistance was mediated by the enzyme OXA-23 in most isolates. These results are in agreement with the literature, which reported the spread of

OXA-23-producing *Acinetobacter* strains in various locations worldwide and the predominance of this OXA in Brazilian territories, being directly responsible for the high rates of carbapenem resistance[8,19-21]. The relationship between OXA-23 production and imipenem-resistance ratio among the isolates in both hospitals was also observed. The isolates positive for $bla_{\text{OXA-23}}$ were also resistant to imipenem, except one isolate.

Furthermore, the high prevalence of $bla_{\text{OXA-51}}$ positive isolates detected in this study showed that *A. baumannii* was the most frequent species, since the oxacillinase is intrinsic in this species[6,22]. It is noteworthy that, although some studies have described the occurrence of $bla_{\text{OXA-51}}$ in *A. nosocomialis*, this still appears to be a rare event[22].

A carbapenem-resistant isolate was positive only for $bla_{\text{OXA-51}}$. The reduced susceptibility to carbapenems in this non-OXA-23 isolates may be mediated by $bla_{\text{OXA-51}}$ overexpression due to the association with the IS element[23]. One carbapenem-susceptible isolate was positive for $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$. This result may be explained by the absence of IS element upstream in the $bla_{\text{OXA-23-like}}$[24,25]. However, susceptible carbapenem isolates carrying $bla_{\text{OXA-23}}$ are considered as silent reservoirs of this gene and can be the source of their spread in a nosocomial environment[26].

In this study, bla_{OXA} genes other than $bla_{\text{OXA-23}}$ and $bla_{\text{OXA-51}}$ were not found, but a $bla_{\text{OXA-72}}$-positive *Acinetobacter* clinical isolate was obtained for the first time from a public hospital in Niterói, Rio de Janeiro. The increasing occurrence of OXA-72-positive *Acinetobacter* isolates in Brazil and OXA-143-producing isolates highlights the importance of continuous epidemiological surveillance to help prevent the dissemination of these organisms[9,18].

A wide varieties of clonal lineages of $bla_{\text{OXA-23}}$ *A. baumannii* isolates causing hospital outbreaks has been reported in various studies in Brazil[27,28]. In this study, genotype A that was detected previously was observed in 5 of the 25 OXA-23-positive strains from Hospital 1 analyzed by PFGE. These results indicate that this strain circulates in the hospital. Some of the OXA-23-producing clones disseminated in Brazil as the clones belonging to ST79 persist and disseminate in the hospital environment[18]. In this study, we did not perform Multilocus Sequence Type MLST analysis of the OXA-23-positive isolates; therefore, our conclusions about these results obtained are limited.

Among the 13 OXA-23-positive isolates from Hospital 2 that was analyzed by PFGE, a multidrug-resistant predominant genotype (N) was detected, which included 46.2% of the isolates. This result suggests that the intra-hospital spread of this particular clonal group may have contributed to the high rate of carbapenem resistance observed in this institution.

Isolates with unique profiles were detected in both hospitals, indicating that in addition to clonal spread, $bla_{\text{OXA-23}}$ was also acquired through horizontal spread in the investigated population. Studies have shown that the dissemination of carbapenemase-producing isolates appears to be due to clonal dissemination; however, studies have also highlighted the horizontal spread of $bla_{\text{OXA-23}}$[29,30].

In conclusion, carbapenem resistance mediated by OXA-23 was high, with most isolates being resistant to carbapenems. A high genetic diversity was verified among the OXA-23-positive isolates analyzed, with the occurrence of both clonal and horizontal dissemination of $bla_{\text{OXA-23-like}}$. These results suggest a need for continuous epidemiological surveillance studies to assist in the control of the dissemination of these carbapenem-resistant strains in the investigated hospitals.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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